

NUCLEIC ACIDS FOR DETECTING *ASPERGILLUS* AND OTHER FILAMENTOUS FUNGI

Abstract

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Nucleic acids for detecting *Aspergillus* species and other filamentous fungi are provided. Unique internal transcribed space 2 coding regions permit the development of nucleic acid probes specific for five different species of *Aspergillus*, three species of *Fusarium*, four species of *Mucor*, two species of *Penicillium*, five species of *Rhizopus*, one species of *Rhizomucor*, as well as probes for *Absidia corymbifer*, *Cunninghamella elagans*, *Pseudallescheria boydii*, and *Sporothrix schenkii*. Methods are disclosed for the species-specific detection and diagnosis of infection by *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizomucor*, *absidia*, *Cunninghaemella*, *Pseudallescheria* or *Sporthrix* in a subject. Furthermore, genus-specific probes are also provided for *Aspergillus*, *Fusarium* and *Mucor*, in addition to an all-fungus nucleic acid probe.

In the claims:

Please amend the claims as follows:

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1. (Amended) An isolated nucleic acid probe that consists essentially of 10 to 50 consecutive nucleotides for species-specific identification of *Fusarium*, wherein the probe hybridizes to the internal transcribed spacer 2 nucleic acid sequence of *Fusarium solani* (SEQ ID NO:6), or *Fusarium moniliforme* (SEQ ID NO:7), but does not hybridize to the internal transcribed space 2 nucleic acid sequence of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO:10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsporus* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18), *Rhizopus stolonifer* (SEQ ID NO: 19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), or *Sporothrix schenkii* (SEQ ID NO:29) .

Please cancel claims 2-6, without prejudice.

ad 7. (Amended) The isolated nucleic acid probe of Claim 1 wherein the probe selectively hybridizes with a *Fusarium solani* nucleic acid of SEQ ID NO:6, or a complementary sequence thereof.

8. (Amended) The isolated nucleic acid probe of Claim 1 wherein the probe selectively hybridizes with a *Fusarium moniliforme* of SEQ ID NO:7, or a complementary sequence thereof.

Please cancel claims 9-23, without prejudice.

ad 24. (Amended) A method of detecting a species of *Fusarium solani* (SEQ ID NO:6) or *Fusarium moniliforme* (SEQ ID NO:7), in a sample comprising contacting the sample with a nucleic acid probe consisting essentially of 10 to 50 consecutive nucleotides that selectively hybridizes with a nucleic acid having a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6, or a complementary sequence thereof; wherein hybridization of the nucleic acid probe with the sample indicates the detection of the *Fusarium* species in the sample.

ad 25. Please cancel claims 25-29, without prejudice.

ad 30. (Amended) The method of Claim 24, wherein the probe selectively hybridizes with a *Fusarium solani* nucleic acid of SEQ ID NO:6, or a complementary sequence thereof.

31. (Amended) The method of Claim 24, wherein the probe selectively hybridizes with a *Fusarium moniliforme* of SEQ ID NO:7, or a complementary sequence thereof.

Please cancel claims 34-46.

ad 47. (Amended) An isolated nucleic acid probe for identifying a member of a *Fusarium* genus wherein the probe consists essentially of a nucleotide sequence as set forth as SEQ ID NO:59, or a complementary sequence thereof, respectively.

Please cancel claim 48.

49. (Amended) A method for detecting a member of a *Fusarium* genus in a sample, comprising

08 combining the sample with a nucleic acid probe that selectively hybridizes with a portion of the nucleic acid of SEQ ID NO:59, or a complementary sequence thereof, respectively, wherein hybridization of the probe with the sample indicates the presence of *Fusarium* in the sample.

Please add the following new claims:

51. (New) An isolated nucleic probe that hybridizes to an internal transcribed spacer 2 region of a *Fusarium* species, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51, and wherein the probe does not hybridize to but does not hybridize to the internal transcribed space 2 nucleic acid sequence of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO:10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsporus* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18), *Rhizopus stolonifer* (SEQ ID NO: 19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), or *Sporothrix schenkii* (SEQ ID NO:29).

52. (New) The isolated nucleic probe of Claim 1, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49.

53. (New) The isolated nucleic probe of Claim 1, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:50.

54. (New) The isolated nucleic probe of Claim 1, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:51.

55. (New) The method of Claim 24, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49.

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56. (New) The method of Claim 24, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:50.

57. (New) The method of Claim 24, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:51.

58. (New) A isolated nucleic acid sequence comprising a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6.

59. (New) A isolated nucleic acid sequence consisting essentially of a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6.

REMARKS

This Preliminary Amendment is submitted to enter Applicants' claims of priority from corresponding U.S. Patent Application No. 09/423,233 filed June 27, 2000, which is a § 371 national stage of PCT/US98/08926 filed May 1, 1997, in turn claims the benefit of U.S. Provisional Application No. 60/045,400 filed May 2, 1997. In addition, the specification is amended to include the abstract submitted in the PCT as a separate page.

Claims 1, 7, 8, 24,30-31, 47, and 49 are amended herein to correct form. Support for the amending language of these claims can be found throughout the specification, specifically on page 4, lines 4-22, page 5, lines 16 to page 7, lines 27. New claims 51-59 are added. Support for new claims 51-59 can be found throughout the specification, specifically on page 4, lines 4-22,

page 5, lines 16 to page 7, lines 27, on page 22 (Table 2), and on page 25, line 2 to page 27, line 4.

Claims 2-6, 9-23, 25-29, 34-46, 48, and 50 are canceled solely in order to reduce the filing fees. Applicants reserve the right to pursue the subject matter of these claims in a continuation application.

No new matter is added.

CONCLUSION

If any minor matters remain to be discussed prior to examination, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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